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Indices of antioxidant status in rats subjected to wood smoke inhalation and/or thermal injury

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Abstract

The present study investigated antioxidant status in lung, liver, heart and kidney in a rat model to simulate an inhalation injury as might be encountered by firefighters and burn victims. Anesthetized rats received either a 20% total body surface area (TBSA) full thickness scald or a sham burn. After a 5 h recovery period, half of the animals in the burn or sham burn groups were exposed to cooled western bark (fir and pine) smoke for 16.25 min. The remaining rats in each group breathed room air. At 1, 12, 24, 48 and 96 h after exposure to the smoke, five rats from each of the four groups were euthanatized and lungs were lavaged by infusing three 5 ml aliquots of normal saline for evaluation of airway cellular content and lung wet to dry weight ratios to estimate lung water content. A second series of five rats/group per time point were euthanatized at the above times and lung, liver, kidney and heart were removed for evaluation of tissue antioxidant enzyme activities and for thiobarbituric acid reactive substances (TBARS) concentrations, as well as for lung histology. Smoke exposure resulted in average plasma carboxyhemoglobin (COHb) of $19 \pm 2\%$ in the two smoke exposed groups and produced areas of erosion of the tracheal surface, resulting in loss of epithelium and exposed basement membrane. Lung water content was not significantly different among the four groups during the 96-h experimental period. Lung TBARS levels were 2–3-fold higher at 12 h in smoke exposed rats compared with controls. These levels peaked at 24 h and remained significantly elevated at 48 h compared to controls. TBARS were also elevated in liver, but not in heart or kidney in response to burn or combined injury. Minor effects on lung antioxidant enzyme activities were observed after smoke inhalation. These data suggest that smoke inhalation, independent of burn injury, induces an oxidant stress that persists for at least the first 48 h after smoke exposure.

Keywords: Scald burn; Smoke inhalation; Lung; Liver; Reactive oxygen species

1. Introduction

Inhalation injury, generally resulting from structural fires, is a major comorbid factor in patients with thermal injury, occurring in about 30% of patients with major burns (Herndon et al.,

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1985). It has been reported that patients with combined smoke inhalation and thermal injuries tend to be hemodynamically unstable and have about a 50% higher initial fluid requirement for resuscitation than estimated for the burn surface area alone (Navar et al., 1985; Thompson et al., 1986; Demling et al., 1995). Inhalation injury reportedly accounts for 20–84% of burn mortality and is associated with higher mortality rates for every age and burn size category (Herndon et al., 1985; Thompson et al., 1986; Shirani et al., 1987).

Inhalation of smoke primarily injures the airways and may not produce clinically significant symptoms for 24–72 h (Lalonde et al., 1995). Smoke inhalation induces a number of pathophysiologic responses including impairment of mucocilliary function (Fitzpatrick and Cioffi, 1994), inflammatory responses such as interstitial edema, neutrophil infiltration, generation of oxygen free radicals and pseudomembrane formation (Lalonde et al., 1995; Fitzpatrick and Cioffi, 1994; Youn et al., 1992; Hubbard et al., 1991). Direct alveolar damage, including alveolar edema, may also occur depending on the dose of smoke, the toxicity of the chemicals present in the smoke or as a consequence of surfactant denaturation (Herndon et al., 1985; Fitzpatrick and Cioffi, 1994; Nieman et al., 1995).

At present treatment of smoke inhalation injuries is primarily supportive. Thus, research efforts to elucidate the underlying pathogenesis of smoke inhalation injury could lead to improved therapies to prevent any secondary inflammatory sequelae. Because of the complexity of smoke inhalation and the likelihood of other injuries present in patients, no ideal animal model exists today. The majority of studies in experimental animals have employed large animals, such as sheep, where the animal is anesthetized, and smoke is introduced by mechanical ventilation (Demling et al., 1995; Lalonde et al., 1995; Hubbard et al., 1991; Matsumoto et al., 1994; Tasaki et al., 1997; Ogura et al., 1994a; Kikuchi et al., 1996). Although use of these animals has contributed much to our understanding of smoke inhalation injury, they are too costly to use routinely to screen potential therapeutic modalities that may benefit the smoke inhalation patient. Therefore, a few studies have investigated smoke inhalation in a small animal, such as a rodent, which may be useful for the evaluation or screening of potential therapeutic strategies. In our interest to further explore the effects of smoke inhalation on the generation of reactive oxygen species, the present study evaluates the time course aspects of the antioxidant defense system over a 96-h period after smoke inhalation in various tissues of rats. Since we have significant experience with the effects of burn injury in rats at our institute, the present study also investigated smoke inhalation in combination with a cutaneous scald burn.

2. Materials and methods

2.1. Experimental design

Adult, male Sprague–Dawley rats, weighing initially 382 ± 2 g, were anesthetized with pentobarbital and randomly assigned to four groups. A section of the back of each rat, corresponding to 20% of the total body surface area (TBSA), was shaved. These rats then received either a full thickness scald or a sham burn to that portion of the back using the template described by Walker and Mason (1968). A 20% TBSA scald burn was induced by dipping the rat in 100 °C water for 10 s (Brown et al., 1976). Sham burn rats were dipped in water at room temperature. After a 5 h recovery period when the animals were awake from anesthesia, half of the animals in the burn or sham burn groups were exposed to cooled western bark (fir and pine) smoke for 16.25 min. The remaining rats in each group breathed room air. Thus the four groups were as follows: (1) control (sham burn, air); (2) sham burn, smoke exposed; (3) scald burn, smoke exposed; and (4) scald burn, air. Rats were returned to their cages, allowed food and water ad libitum, and one set of five rats/group/time point was euthanatized by an overdose of pentobarbital at 1, 12, 24, 48 or 96 h after smoke inhalation for determining lung wet-to-dry weight ratios to estimate lung water content, and measuring lung, liver, heart and kidney antioxidant enzyme activities, and thiobarbituric

acid reactive substances (TBARS) as an index of lipid peroxidation. Blood was removed in rats killed at 1 h after smoke inhalation for measurement of carboxyhemoglobin (COHb) concentrations according to standard blood gas technique using an IL1303 blood gas analyzer (Instrumentation Laboratories, Inc., Lexington, MA). A second set of five rats/group/time point were anesthetized with pentobarbital and gently lavaged with three 5 ml aliquots of normal saline for determination of bronchoalveolar lavage fluid protein and blood cell counts. Airways and lung were harvested for preparation and analysis by scanning electron microscopy (SEM) as previously described (Hubbard et al., 1991). Lung parenchyma was also processed for hematoxylin/eosin staining. At each time point, when analyses were not performed with fresh tissue, the tissue samples were frozen at -70 °C for later analysis within 3 months.

2.2. Tissue analyses

Lung wet-to-dry weight ratios were determined by standard technique compensating for the presence of blood in the lung tissue, as previously described (Ogura et al., 1994a).

Bronchoalveolar lavage fluid was analyzed for blood cell counts by standard clinical methodologies using a cell counter (Coulter, Fullerton, CA). Protein concentrations in lavage fluid or tissue homogenates were determined by a commercial kit (BioRad Laboratories, Richmond, CA).

Lung, liver, heart and kidney were homogenized in 50 mM potassium phosphate buffer, pH 7.4. Malondialdehyde concentrations were determined as TBARS in the butanol phase using 1,1,3,3-tetraethoxypropane as standard, as described by the method of Naito et al. (1993). Glutathione peroxidase (GP) and glutathione reductase (GR) activities were determined spectrophotometrically by following the oxidation of NADPH (Lawrence and Burk, 1976; Rogers and Augusteyn, 1978). Total superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund (1974). Mn-SOD was determined under the same conditions except the buffer contained 1 mM KCN. CuZn-SOD was determined by subtracting Mn-SOD activity from the total activity.

Specimens for both SEM and transmission electron microscopy (TEM), respectively were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 at 4 °C for 24 h, then placed in buffer (Hubbard et al., 1991). The specimens were post-osmicated for 1 h in 2% osmium tetroxide. SEM specimens were dehydrated in graded ethanol–water solutions to absolute ethanol and then placed in a 1:1 mixture of ethanol and Peldri II for 1 h. Sublimation of the specimens was carried out according to the method of Kennedy et al. (1989). Dried specimens were coated in a DC sputter-coater with 20 nm of gold/palladium and examined in a Phillips XL20 scanning electron microscope at an accelerating voltage of 10 kV. Lung specimens for TEM were routinely processed, sectioned and examined in a Philips 400T transmission electron microscope. All specimens were viewed without identification of their treatment group.

2.3. Statistical analysis

Data are presented as mean $\pm$ SE. Data were analyzed by two-way Analysis of Variance with treatment and time as the independent variables. If a significant F-statistic was determined, data were further analyzed by Tukey HSD test. A $P < 0.05$ was considered statistically significant.

3. Results

Neither smoke exposure nor scald burn significantly affected final body weights over the 96 h experimental period (data not shown). Smoke exposure resulted in significant levels of COHb compared to air-exposed controls. COHb concentrations in smoked rats without or with scald burn were 16.8 ± 1.8 and 21.5 ± 2.2%, respectively, whereas these values were essentially zero in rats exposed to air. Differences in COHb concentrations between the two smoked groups were not statistically significant.

3.1. Lavage fluid

Total white blood cell counts in lavage fluid were not significantly different among the four groups at any time throughout the 96 h experimental period (Table 1). Similar observations were made with respect to red blood cell counts in lavage fluid (Table 1). Protein levels in lavage fluid from scald burn or control rats were undetectable. In contrast, protein was detected in lavage fluid from both groups of rats exposed to wood smoke (Table 1). Measurable levels of protein were detected in lavage fluid from smoke-exposed rats throughout the 96 h experimental period, whereas protein was only detected in the combined burn/smoke group for the first 24 h. The highest protein levels were seen 1 h after smoke exposure, but the values did not achieve statistical significance compared to the other times.

3.2. Lung tissue

Lung water content did not differ significantly among the four groups of rats throughout the 96 h experimental period (Table 2).

Smoke inhalation and/or burn injury significantly elevated lung TBARS levels 2–3-fold by 12 h after injury when compared with controls (Fig. 1). TBARS were slightly higher in the combined injury group when compared to either smoke or burn alone, but the differences were not statistically significant. Lung TBARS levels were highest 24 h after injury and remained significantly higher than controls at 48 h. Except for the combined injury group, TBARS levels were not significantly different from controls at 96 h (Fig. 1). Liver TBARS were significantly higher than controls in the burn or combined injury groups at 12 and 24 h after smoke exposure but the magnitude of increase was less than in lung (Table 3). Liver TBARS were about 50% higher than controls in all injury groups at 48 h, but returned to control levels by 96 h. Heart and kidney TBARS concentrations were not significantly different among groups at any time during the study (data not shown).

Lung Mn-SOD activity was not significantly different among all groups at 1 h after injury (Fig. 2). At 12 h after injury, Mn-SOD activity was 38% lower in the smoke-exposed rats than in controls. Enzyme activity was generally 10–20%

Table 1
Effect of smoke inhalation and/or burn injury on total lavage fluid protein and blood components

Time (h)		Control	Smoke	Burn and smoke	Burn
1	Protein (μg/ml)	ND[a]	0.146 ± 0.058	0.078 ± 0.008	ND
	RBC (10^9 cells/ml)	0.044 ± 0.005	0.043 ± 0.007	0.028 ± 0.004	0.034 ± 0.004
	WBC (10^6 cells/ml)	1.6 ± 0.14	1.8 ± 0.15	1.5 ± 0.19	1.98 ± 0.23
12	Protein	ND	0.089 ± 0.01	0.046 ± 0.011	ND
	RBC	0.042 ± 0.007	0.048 ± 0.007	0.036 ± 0.006	0.054 ± 0.004
	WBC	2.4 ± 0.12	3.02 ± 0.4	2.1 ± 0.2	2.48 ± 0.47
24	Protein	ND	0.057 ± 0.024	0.053 ± 0.022	ND
	RBC	0.046 ± 0.005	0.064 ± 0.011	0.053 ± 0.004	0.042 ± 0.005
	WBC	2.4 ± 0.22	2.76 ± 0.5	2.0 ± 0.34	2.34 ± 0.31
48	Protein	ND	0.035 ± 0.013	ND	ND
	RBC	0.04 ± 0.005	0.058 ± 0.004	0.054 ± 0.002	0.05 ± 0.003
	WBC	2.6 ± 0.19	1.8 ± 0.14	2.16 ± 0.11	2.36 ± 0.22
96	Protein	ND	0.07 ± 0.034	ND	ND
	RBC	0.03 ± 0.003	0.064 ± 0.009	0.03 ± 0.002	0.044 ± 0.004
	WBC	1.8 ± 0.25	2.9 ± 0.27	3.00 ± 0.17	2.28 ± 0.23

Data expressed as mean ± SE for five animals/group.
[a] ND, not detectable.

Table 2
Effect of smoke inhalation and/or burn injury on lung water content

Time (h)	Time control	Smoke	Burn and smoke	Burn
1	4.15 ± 0.07	4.02 ± 0.11	4.02 ± 0.02	3.88 ± 0.03
12	4.02 ± 0.05	3.96 ± 0.10	3.86 ± 0.05	3.72 ± 0.18
24	3.95 ± 0.04	4.40 ± 0.47	4.23 ± 0.20	4.04 ± 0.08
48	4.24 ± 0.21	3.97 ± 0.08	4.24 ± 0.13	4.27 ± 0.14
96	4.12 ± 0.08	4.05 ± 0.10	4.31 ± 0.12	4.16 ± 0.06

Data expressed as mean ± SE for five animals/group.

lower than controls from 12 to 48 h after injury, but these differences did not achieve statistical significance (Fig. 2). Liver Mn-SOD activities in the smoked or burned rats were significantly lower than controls at 12 h, but enzyme activity in all injured groups were at control levels at the other experimental times. Lung CuZn-SOD activity was significantly lower in burned rats than controls and combined injury groups at 12 and 24 h after smoke inhalation (Fig. 2). Smoke exposure did not significantly affect lung CuZn-SOD activity. Liver CuZn-SOD activity was only significantly lower than control in all injury groups at 1 h after smoke inhalation (Table 3).

Lung GP activity was significantly higher at 24 h in the smoked and combined injury groups when compared with controls (Fig. 3). At 48 h, enzyme activities in the burn and combined injury groups were significantly lower than controls. Enzyme activities in all injured groups returned to control levels by 96 h (Fig. 3). Liver GP activity was not significantly different from control throughout the experimental period (Table 3). No statistically significant differences in lung or liver GR activities were observed at any time after injury among the four groups (Fig. 3, Table 3). Antioxidant enzyme activities were not significantly different than controls in heart and kidney throughout the study (data not shown).

3.3. Electron microscopy observations

SEM of control rat tracheal epithelium did not reveal any abnormal morphology throughout the 96 h experimental period. In contrast, SEM of tracheal epithelium from smoked animals revealed moderate cellular erosion, some pseudomembrane formation and matting of cilia as early as 12 h following smoke exposure (Fig. 4). These morphologic alterations persisted in smoke-exposed animals at the end of the 96 h experimental period (Fig. 5). Rats with burn injury alone showed much less tracheal damage with some matting of cilia as the primary change and only occasional areas of cellular erosion (Fig. 4). No pseudomembranes were found in these animals. Rats with both smoke and burn injuries showed the same type and degree of changes as seen in the smoke only rats.

H and E staining of the lung did not detect any overt lesions among the groups. In contrast, TEM studies of the lung from smoked only or smoked and burned rats revealed mild swelling of type I parenchymal cells, slight interstitial edema, areas of mild cellular hyperplasia, and a small amount of proteinaceous material in the alveolar space (data not shown). No abnormalities were observed in lung from the control or burn only groups throughout the 96 h experimental period.

4. Discussion

It is well recognized that carbon monoxide is one of the more dangerous constitutents of smoke and plasma COHb levels exceeding 50% are considered evidence for carbon monoxide related deaths in humans (Fitzpatrick and Cioffi, 1994). In the present study average plasma COHb levels in smoke-exposed rats ranged from 16.8 to 21.5% 1 h after smoke exposure. In contrast, in the sheep model in which a moderate dose of smoke is delivered through a ventilator, plasma COHb levels of 28–71% were noted either immediately or at

1 h after exposure (Demling and Lalonde, 1990a; Demling et al., 1995; Ogura et al., 1994b). However, others have reported COHb levels as high as 90% after severe smoke exposure in the sheep models (Sugi et al., 1990). Although COHb may be useful to compare smoke exposure among groups, the literature suggests that in animal models, COHb may not be related to the severity of smoke injury. In the present study, rats were exposed to smoke for 16.25 min without mortality. The smoke was mixed 1:1 with 100% oxygen to reduce the development of hypoxia. However, when exposure was increased by another 15 s to 16.5 min, a 30% mortality rate was observed in smoke-exposed animals, presumably due to an induced acute hypoxia. Thus, smoke exposure in the present study was at or near the maximum tolerated levels.

To assess possible biomarkers to analyze the degree of inhalation injury in the current study, bronchoalveolar lavage fluid was collected. In the current study, protein was only detectable in lavage fluid from animals exposed to smoke. Protein levels were higher and persisted longer in smoked animals in comparison with animals who received a scald burn prior to smoke exposure, but in the first 24 h after exposure, the differences were not statistically significant. Smoke inhalation is associated with early sloughing of bronchoepithelial cells into airways (Abdi et al., 1990) and recruitment of inflammatory cells (Fitzpatrick and Cioffi, 1994). These responses probably account for the protein detected in lavage fluid, but further studies are necessary to identify the cell types and to characterize this response.

Previous studies have also shown that smoke inhalation is primarily a bronchoepithelial injury with no injury occurring to the vascular endothelium (Ogura et al., 1994a). In the present study, lavage fluid red blood cell counts were not significantly different among the groups at all time points examined. These data would suggest that no airway or lung vascular endothelial injury was observed that would have resulted in bleeding into the alveolar spaces.

The present study also did not show an increase in lavage fluid white blood cells in smoke-exposed animals compared with the other groups. Others, however, have reported that infiltration of neutrophils into airways was an early event in the inflammatory process associated with smoke inhalation (Hubbard et al., 1991). These studies have also attributed activation of polymorphonuclear leukocytes (PMN) as involved in injury to the lung parenchyma after smoke inhalation (Traber et al., 1986; Linares et al., 1989; Hubbard et al., 1991). Perhaps the lack of significant injury to the lung, itself, in the present study is consistent with the absence of significant leukocyte infiltration observed. In addition it has also been reported that injury distal to the lung that involves

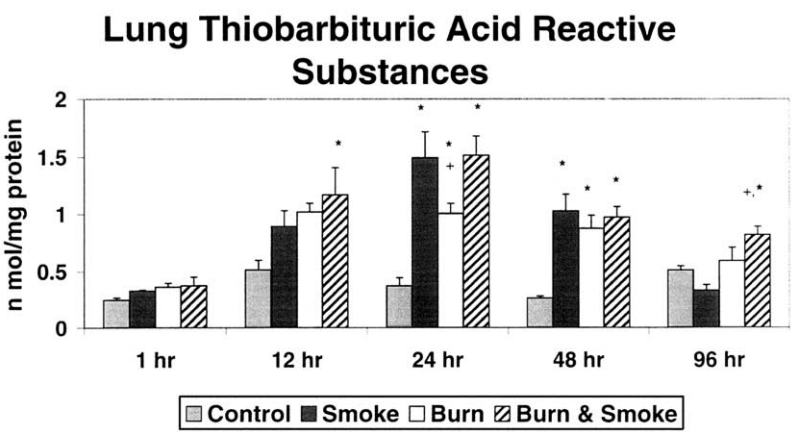

Fig. 1. TBARS levels in lungs from rats subjected to smoke inhalation and/or burns. Data represent mean ± standard error from five animals/group. *$P < 0.05$ from control. $^{+}P < 0.05$ from combined injury group.

Table 3
Effects of smoke inhalation and/or burn injury on rat liver antioxidant enzymes and TBARS

Time (h)	Time control	Smoke	Burn and smoke	Burn
Glutathione peroxidase (*U/mg protein*)				
1	0.335 ± 0.009	0.298 ± 0.020	0.313 ± 0.019	0.316 ± 0.007
12	0.346 ± 0.021	0.317 ± 0.017	0.339 ± 0.011	0.311 ± 0.010
24	0.323 ± 0.015	0.319 ± 0.010	0.320 ± 0.028	0.309 ± 0.013
48	0.340 ± 0.012	0.313 ± 0.004	0.310 ± 0.013	0.326 ± 0.021
96	0.353 ± 0.007	0.365 ± 0.015	0.323 ± 0.022	0.356 ± 0.016
Glutathione reductase (*U/mg protein*)				
1	0.077 ± 0.003	0.071 ± 0.005	0.073 ± 0.004	0.069 ± 0.002
12	0.065 ± 0.003	0.064 ± 0.006	0.065 ± 0.004	0.060 ± 0.005
24	0.050 ± 0.003	0.051 ± 0.001	0.045 ± 0.005	0.046 ± 0.005
48	0.055 ± 0.002	0.056 ± 0.001	0.055 ± 0.003	0.056 ± 0.003
96	0.058 ± 0.003	0.051 ± 0.004	0.054 ± 0.003	0.002 ± 0.054
Mn-SOD (*U/mg protein*)				
1	2.06 ± 0.12	2.19 ± 0.23	2.10 ± 0.19	1.98 ± 0.09
12	2.68 ± 0.08	2.21 ± 0.13*	2.39 ± 0.14	2.22 ± 0.12*
24	1.89 ± 0.11	1.94 ± 0.15	1.96 ± 0.19	1.89 ± 0.12
48	1.81 ± 0.21	1.69 ± 0.09	2.07 ± 0.10	2.00 ± 0.27
96	2.22 ± 0.13	2.16 ± 0.12	2.17 ± 0.16	2.36 ± 0.11
CuZn-SOD (*U/mg protein*)				
1	8.69 ± 0.40	7.80 ± 0.30*	7.17 ± 0.44	7.53 ± 0.22
12	9.27 ± 0.15	9.75 ± 0.35	8.53 ± 0.54	8.38 ± 0.28
24	8.24 ± 0.15	8.17 ± 0.24	7.85 ± 0.32	7.43 ± 0.52
48	8.93 ± 0.62	8.11 ± 0.31	7.24 ± 0.51	7.68 ± 0.55
96	10.41 ± 0.24	9.23 ± 0.72	8.03 ± 0.36	9.59 ± 0.53
Thiobarbituric acid reactive substances (*nmol/mg protein*)				
1	0.214 ± 0.026	0.288 ± 0.027	0.277 ± 0.012	0.261 ± 0.01
12	0.275 ± 0.027	0.339 ± 0.02	0.378 ± 0.017*	0.351 ± 0.013*
24	0.214 ± 0.015	0.247 ± 0.02	0.283 ± 0.012*	0.283 ± 0.017*
48	0.199 ± 0.007	0.322 ± 0.016*	0.34 ± 0.012*	0.325 ± 0.013*
96	0.237 ± 0.005	0.272 ± 0.008	0.268 ± 0.016	0.255 ± 0.014

Data expressed as mean ± SE ($n = 5$).
* $P < 0.05$ from controls.

PMN activation, such as thermal injury, can worsen lung injury after smoke inhalation (Matsumoto et al., 1994). Therefore, based on these observations and the findings of others (Traber et al., 1986), it is surprising that we did not detect significantly greater evidence of white blood cells in lung from the combined burn and smoke group compared with the smoke exposed rats in this study. Perhaps this may relate to the extend of smoke exposure in rats compared to sheep where the smoke is delivered deeper into the lung and/or the greater COHb levels observed in those studies compared with the present one. However, in the present study we did not fractionate the white cells so further work is necessary to determine whether the white cell population changes over time after smoke inhalation injury.

Related, at least in part, to neutrophil infiltration, is the observation of higher levels of indices of lipid peroxidation and oxidative stress in cotton smoke-exposed animals (Youn et al., 1992; Tasaki et al., 1997; Demling and Lalonde, 1990a; Demling et al., 1994; Lalonde et al., 1994). In our own preliminary studies, lavage fluid collected from rats 1 h after wood smoke exposure showed

a 46% reduction in ascorbic acid levels compared with controls (Dubick et al., 1998). In addition, other studies have shown that antioxidants or iron chelators were able to reduce evidence of lipid peroxidation following wood smoke exposure in experimental animals (Zhao, 1990; Nguyen et al., 1995; Demling et al., 1996). In sheep, smoke inhalation resulted in higher malondialdehyde concentrations in liver, but not lung, 24 h after exposure (Demling and Lalonde, 1990a; Demling et al., 1994). In rats exposed to 20 ml/kg smoke, malondialdehyde concentrations were significantly higher in lung, liver and kidney at 24 h post-exposure in comparison with controls (Lalonde et al., 1994). In addition, catalase activity in these organs was significantly lower than controls (Lalonde et al., 1994). The results of the present study generally agree with these observations. Increased liver TBARS in the present study agree with previous observations (Lalonde et al., 1994) and suggests some systemic effects albeit mild. It should also be noted that an oxidative stress has also been associated with burn injury (Sasaki et al., 1983; Demling et al., 1989; Demling and Lalonde, 1990b). In the present study, significantly elevated lung TBARS concentrations were

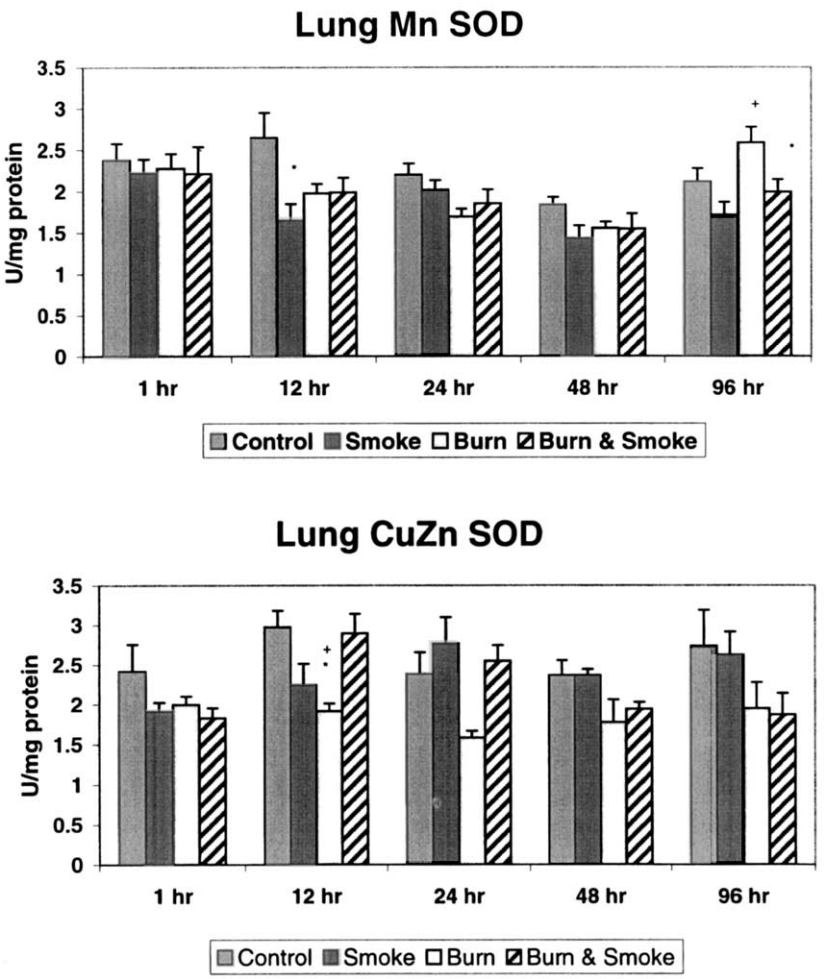

Fig. 2. Mn- and CuZn-SOD activities in lungs from rats subjected to smoke inhalation and/or burns. Data represent mean ± standard error from five animals/group. $^{*}P < 0.05$ from control. $^{+}P < 0.05$ from combined injury group.

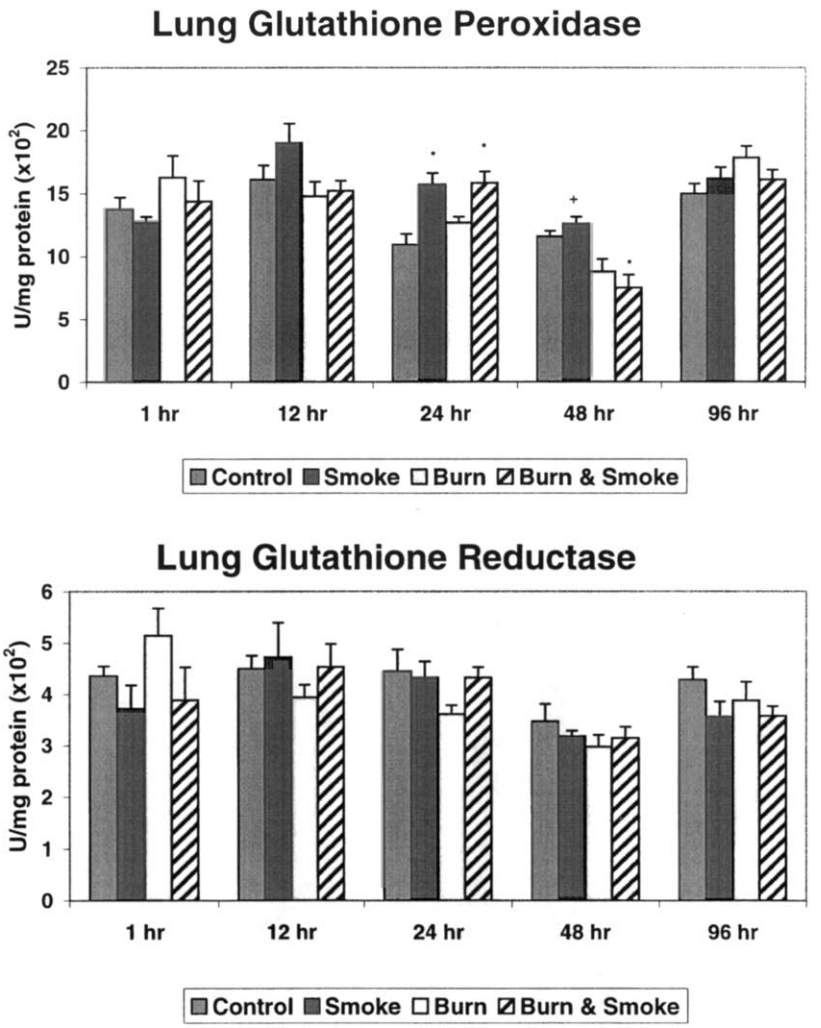

Fig. 3. GP and GR activities in lungs from rats subjected to smoke inhalation and/or burns. Data represent mean ± standard error from five animals/group. $^{*}P < 0.05$ from control. $^{+}P < 0.05$ from combined injury group.

observed in rats subjected to smoke inhalation, burn or combined injury, by 12 h after smoke exposure and the effect persisted for at least 48 h. In addition, some changes in lung antioxidant enzyme activities were also observed in burned and/or smoked rats 12 and 24 h after injury. Taken together, these data are in agreement with previous studies that smoke inhalation and burn injury induce an oxidative stress, and that the injury develops over time. Questions remain regarding the source of oxidants after smoke inhalation.

Inhalation injury has been described to involve both the tracheobronchial tree as well as the lung parenchyma, depending on severity (Fitzpatrick and Cioffi, 1994; Hubbard et al., 1991). In animal models of combined burn and smoke inhalation injury, it has been shown that smoke inhalation preceding burn injury results in greater lung damage than if the burn injury occurred first (Matsumoto et al., 1994). It has been hypothesized that this may be due to greater availability of neutrophils to infiltrate into lung if the smoke inhalation occurs first (Matsumoto et al., 1994). In

reality, this combined injury occurs nearly spontaneously in burn victims, but such a procedure cannot be realistically developed in the laboratory. In the current study, due to logistic constraints, the burn injury was induced prior to smoke inhalation. Although we saw evidence of oxidative stress and morphologic evidence of airway damage, it is possible more severe injury may have been observed had the smoke inhalation occurred prior to burn as has been observed by others (Matsumoto et al., 1994).

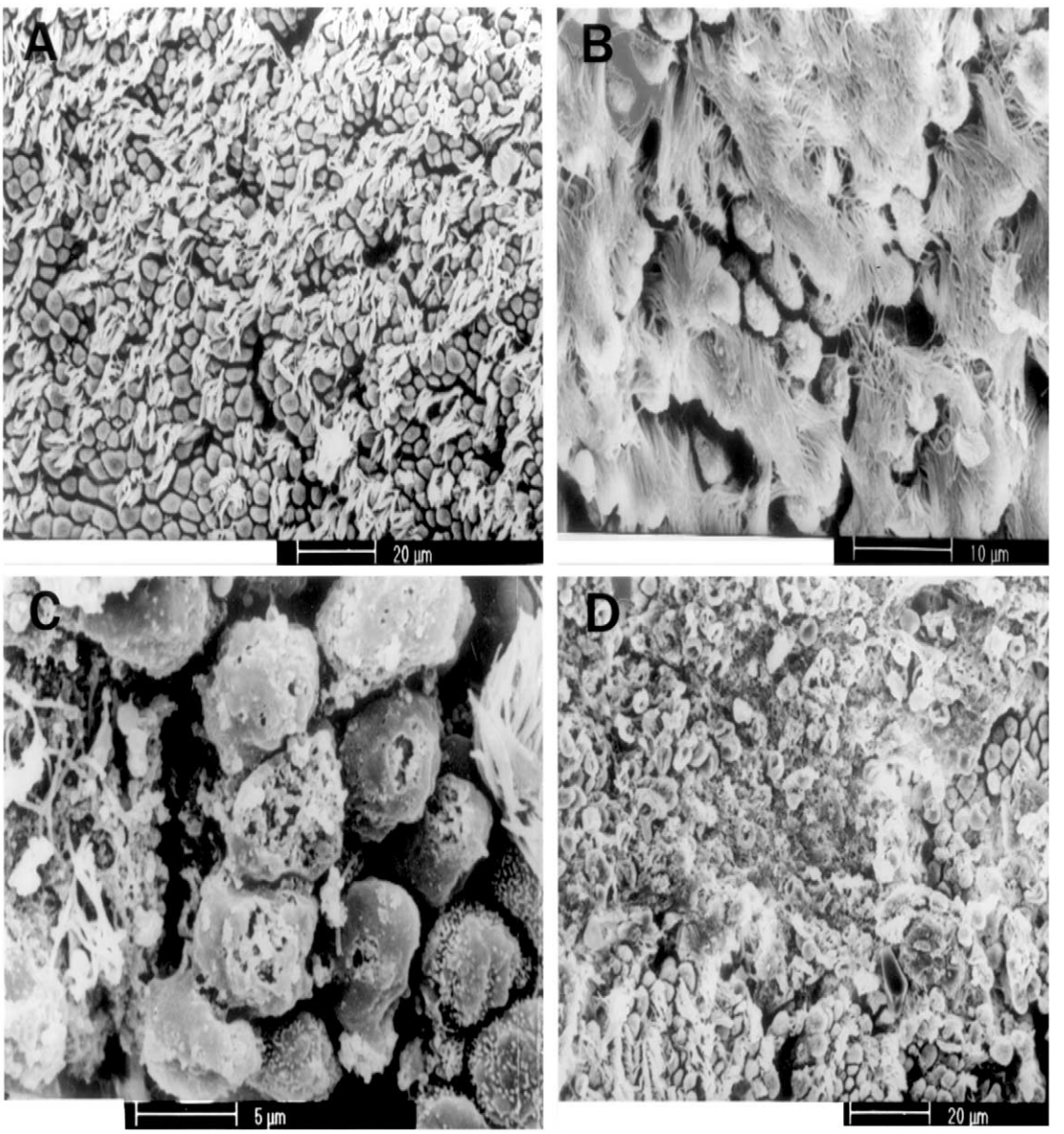

Fig. 4. SEM of rat tracheas at 24 h after injury. (A) Control shows normal tracheal epithelium with both ciliated and non-ciliated cells, × 800. (B) Burn only. Group shows some cilial matting, × 2000. (C) Combined smoke and burn. Trachea reveals cellular erosion, × 4000. (D) Smoke only. Cellular erosion and pseudomembrane formation (arrow) observed, × 800.

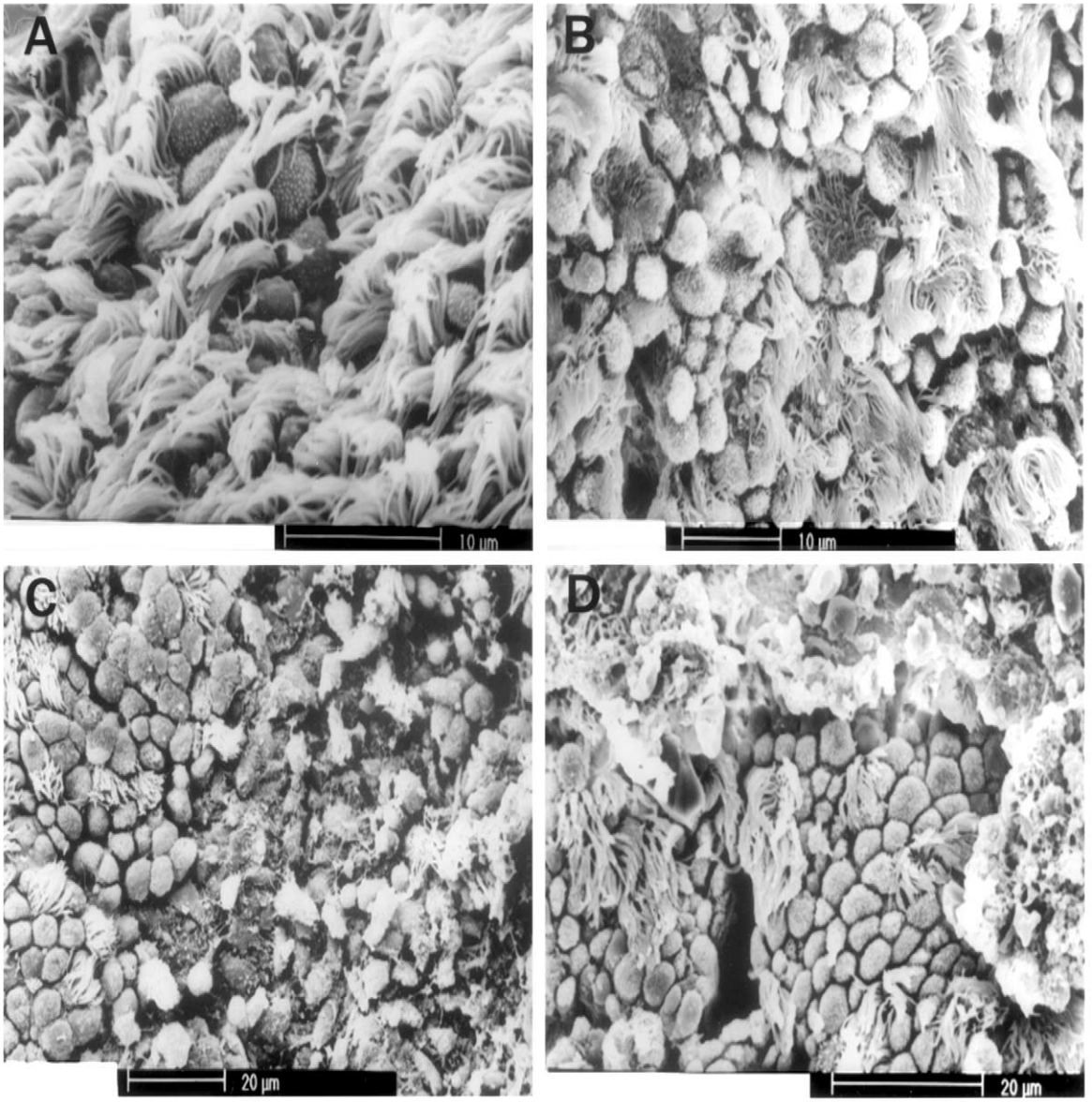

Fig. 5. SEM of rat tracheas at 96 h after injury. (A) Control shows normal appearing epithelium, × 3200. (B) Burn only. Cilial matting less than at 24 h, × 2000. (C) Combined burn and smoke. Trachea continues to show large area of cellular erosion, × 1000. (D) Smoke only. Persisting pseudomembranes (arrow) observed, × 1600.

In summary, in this combined model of burn injury and smoke inhalation in spontaneously breathing rats, evidence of oxidative stress and histologic damage to airways was observed as early as 12 h following injury and persisted throughout the experimental study although most of the biochemical indices of oxidative injury returned to control levels by 96 h. In this model, rats subjected to combined burn and smoke inhalation did not show a consistently worse injury to the lung than rats subjected to smoke inhalation alone. Nevertheless, these studies suggest that

the use of antioxidants may be a worthwhile avenue of therapy and is worthy of further exploration in the treatment of smoke inhalation injury.

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